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Estimating realized heritability for growth in Zhikong scallop (*Chlamys farreri*) using genome-wide complex trait analysis

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Abstract

In selective breeding, a central parameter in summarizing the proportion of variance due to genetics for the purpose of predicting gains from selection is realized heritability (h^2). We applied the genome-wide complex trait analysis (GCTA) method to genome-wide SNP data obtained by 2b-RAD reduced-representation genotyping and phenotypic data for four traits (shell length, shell height, shell width and whole wet weight) with the aim of establishing the heritability for growth in Zhikong scallop (*Chlamys farreri*) in a selective breeding program. The GCTA-based heritabilities of 0.42 (S.E. 0.09) for shell length, 0.47 (S.E. 0.07) for shell height, 0.54 (S.E. 0.11) for shell width and 0.28 (S.E. 0.03) for whole wet weight, which were estimated with uncommon SNPs (26,471 SNPs with MAF >2%), were close to and strongly correlated (r = 0.957) with traditional estimates of realized heritability and in the moderate-to-high range, in line with values previously obtained for growth rates in bivalves. h^2_{GCTA} with 20,000 and 10,000 SNPs were very close to estimates with uncommon SNPs and appeared relatively robust to SNP number. Removing causal SNPs had little effect on obtaining reliable estimates of h^2_{GCTA}, suggesting that causal SNPs is not necessary for accurate estimates of h^2. Chromosome-wise heritability estimates suggested that the genetic contribution to growth complex traits is scattered across the genome and driven by many loci with small effect rather than a few causal loci with a large effect. These results indicate that the genome-wide complex trait analysis method may be useful for estimating realized heritability for growth in Zhikong scallop with SNPs obtained by

reduced-representation genotyping approaches, which is less expensive and faster than full-genome sequencing for non-model species growing in natural environments.

Keyword: Selective breeding, Heritability, 2b-Rad, SNP, GCTA, Zhikong scallop, *Chlamys farreri*

1. Introduction

Selective breeding is a common and effective approach for genetic improvement of aquaculture stocks. Many studies have been carried out to estimate the quantitative genetic variance of important traits in bivalve molluscs for the purpose of genetic improvement from selection. A central parameter in estimating responses to selection and summarizing the proportion of variance due to genetics is heritability (h^2) (Wright, 1920; Falconer and Mackay, 1996). In scallops, most selection experiments on growth rate and/or live weight were successful in changing the selected trait in the desired direction. Significant heritabilities from selection for growth were reported in the Catarian scallop (*Argopecten ventricosus*) (Ibarra et al., 1999), the Japanese scallop (*Patinopecten yessoensis*) (Liang et al., 2010) and the Bay scallop (*Argopecten irradians irradians*) (Stiles et al., 1998; Zheng et al., 2006). The Zhikong scallop (*Chlamys farreri*) naturally distributes along the seacoasts of China, Japan and Korea, and is a commercially important bivalve species in China. Currently, genetic studies focusing on scallop growth, reproduction and immunity represent active research directions. Genetic and genomic resources for Zhikong scallop have rapidly expanded, including low-density linkage (Wang et al., 2005), physical (Jiao et al., 2014), and cytogenetic maps (Zhang et al., 2008), fosmid and bacterial artificial chromosome (BAC) libraries (Zhang et al., 2007).

Traditionally, heritability can be estimated from the ratio of the observed selection response (*R*) to the observed selection differential (*S*), which is equivalent to narrow-

sense heritability (often called realized heritability) in selective breeding (Wray and Visscher, 2008). In mass-selection experiments, a common method of determining realized heritability is to compare the offspring to the parents at the same age. When the parental population cannot be used as a baseline for determination of improvement, simultaneous control populations are generally substituted (Hadley et al., 1991). Because heritability is dependent upon the environment in which organisms are reared, h^2 estimates made under controlled conditions may not be good estimators of h^2 in natural conditions (Geber and Griffen, 2003). It is considerably more challenging for species that are not amenable to be reared in controlled common environments, especially for scallops. Alternative methods using genome-wide data to estimate heritability based on the proportion of phenotypic variance explained by genotyped SNPs have been developed in the animal breeding literature (Meuwissen et al., 2001; Van Raden, 2008; Goddard and Hayes, 2009; Campos et al., 2012). One of these methods, genome-wide complex trait analysis (GCTA), can estimate the distant genetic relationship between unrelated individuals using SNP data and can correlate the genetic similarity to the phenotypic similarity, thus partitioning the total phenotypic variance into genetic and environmental causes (Yang et al., 2011). If the assayed SNPs adequately capture the relationships among individuals at causative alleles, h^2 estimated by GCTA (h^2_{GCTA}) is equivalent to narrow-sense heritability (Yang et al., 2011).

Nonetheless, one major premise of genomic-based estimates of heritability, such as

that implemented in GCTA, is the requirement of sufficient number of genetic markers. It is difficult for aquacultural breeders to obtain high-density markers at a low cost and this situation is even worse for species with little or no genomic resources. Recent genotyping-by-sequencing (GBS) methods, such as RAD and 2b-RAD (Davey et al., 2011; Wang et al., 2012), collect genome scale polymorphism data through reduced-representation genotyping and can also make precise estimates of heritability practical even for natural populations of long-lived non-model species (Dou et al., 2016). In this study, we applied the GCTA method to genome-wide SNPs obtained by 2b-RAD sequencing with the aim of establishing the realized heritability for growth of Zhikong scallop (*Chlamys farreri*, Jones et Preston 1904) in a selective breeding program. In order to evaluate the performance of the GCTA method with 2b-RAD SNPs in estimating realized heritability of Zhikong scallop, our objectives were to (i) compare the h^2_{GCTA} estimates of heritability with the estimates of realized heritability h^2 obtained from the empirical method in selective breeding, (ii) evaluate how estimates of h^2_{GCTA} are affected by SNP density and minor allele frequency (MAF), (iii) examine the effects excluding putatively causal SNPs has on h^2_{GCTA} estimates and (iv) assess whether the genetic contribution to growth is driven by a few SNPs with a large effect or many SNPs with small effect.

2. Materials and Methods

2.1. Scallop samples and phenotypes

Phenotype data were traditional size-related characters as complex traits, such as shell length, shell height, shell width, whole wet weight. Shell height was measured from the hinge to the opposite end of the shell. Shell length was measured as the maximum dimension at right angles to the height. Shell width was measured as the greatest vertical distance between the two valves.

2.2. Realized heritability estimation

The parental scallops used in this study were collected from a cultured population in Qingdao Shazikou, Shandong Province, China. In February 2012, 1000 scallops for each traits were brought to the hatchery for selection and conditioning. For the parental populations, the selection intensity planned was i =1.755 for the four complex traits. However, the observed selection intensity, which was estimated from the standardized difference between the means of the selected parents from the population divided by the standard deviation of the population (Falconer and Mackay, 1996), was lower for the four traits, 1.651 for shell length, 1.647 for shell height, 1.732 for shell width, and 1.606 for whole wet weight. For each trait, 100 scallops per replicate every time were randomly sampled at 8, 12, 18 and 24 months of age. Mean sizes of selected and control groups were compared at the different age. All comparisons were made using two-tailed t-tests assuming unequal variances (Sokal and Rohlf, 1996).

In the present study, considering the huge variation in the annual growth rate of

Zhikong scallop due to environmental factors, a simultaneous Control group was used. The realized heritability (h^2) was calculated using equation (2) of Hadley et al. (1991) as follows:

$$h^2 = R/S = (\bar{x}_s - \bar{x}_c)/(i * sd_c) \quad (1)$$

where R is response to selection and equals the difference between selected and control means ($\bar{x}_s$ and $\bar{x}_c$), S is the selection differential and may be estimated as the product of the standard deviation of the control offspring and the intensity of selection from a truncated standard normal distribution (sd_c and i).

2.3. 2b-RAD sequencing

2b-RAD libraries were prepared by following the protocol developed by (Wang et al., 2012). For the individuals, standard BsaXI libraries were constructed, whereas for the progenies, reduced representation libraries were constructed using adaptors with 50 -NNT-30 overhangs to target a subset of all BsaXI fragments in the Zhikong scallop (*Chlamys farreri*) genome (http://mgb.ouc.edu.cn/cfbase/html/). A unique barcode was incorporated into each library during library preparation, and then all libraries were pooled for single-end sequencing (1 × 50 bp) using an Illumina GA-II sequencer. All the 2b-RAD sequences were archived in the SRA database (accession no. SRA065207). De novo 2b-RAD genotyping was performed using the RAD typing program v1.0, which is an integrated pipeline that enables both accurate de novo codominant and dominant genotyping in mapping populations (Fu et al., 2013).

2.4. SNP-based heritability estimation

We used GCTA version 1.24.2 (Yang et al., 2011) to estimate the proportion of phenotypic variance explained by the genotyped SNPs. First, GCTA was used to create the genetic relationship matrix (GRM) for estimating the pair-wise genetic relationship between individuals. Then, we applied principal components analysis to calculate the first ten eigenvectors of GRM, which we included as covariates in all the heritability estimation analyses to control for potential population structure. Next, we estimated univariate heritabilites of complex traits of scallop by the restricted maximum likelihood method in GCTA.

We randomly collected 538 individuals at 24 months in the selected groups of four traits and used 2b-RAD sequencing to obtain a high-quality set of SNPs (31,361) with an average calling rate of 84% in this study. Using the physical map of Zhikong scallop (Jiao et al., 2014), the missing genotypes were inferred by the Beagle software (Browning and Browning, 2009). To evaluate how marker density influences heritability estimation, we compared h^2_{GCTA} estimated using uncommon SNPs (26,471 SNPs with MAF $> 2\%$) to h^2_{GCTA} from 100 data sets approximately 20,000, 10,000 and 2,500 randomly sampled SNPs in the uncommon SNP set. To evaluate how common SNPs affect h^2_{GCTA} estimates, we compared h^2_{GCTA} estimated using 11,941 SNPs with MAF $> 10\%$ to h^2_{GCTA} with uncommon SNPs with MAF $> 2\%$. To evaluate whether or not causal Locus affects h^2_{GCTA} estimates, we estimated h^2_{GCTA} using a pruned data set in which we removed 1000 SNPs that a genome-wide

association (GWAS) tool (Purcell et al., 2007) conducted with these same data identified as having the lowest P-values with phenotypic variation in each trait. To assess whether the genetic contribution to variation in each complex traits is driven by a few loci with a large effect or many loci with small effect, we did univariate heritability estimations for each chromosome separately for each complex traits.

3. Results

Growth data of the selected and control groups at 8, 12, 18 and 24 months of age, results of t-tests, responses to selection (R) and realized heritability (h^2) estimates calculated by equation (1) were presented in Table 1. For the four phenotypes we analysed, selected groups did significantly grow faster than controls from 8 to 24 months of age (P<0.001) and h^2 ranged from near 0.25 for whole wet weight to 0.54 for shell width. h^2_{GCTA} estimates conducted using uncommon SNPs (26,471 SNPs with MAF $> 2\%$) and phenotypes from the randomly selected 538 scallops at 24 months in the selected group were 0.42 (S.E. 0.09) for shell length, 0.47 (S.E. 0.07) for shell height, 0.54 (S.E. 0.11) for shell width and 0.28 (S.E. 0.03) for whole wet weight (Table 2). For all the traits, the mean of the realized heritability estimates was highly correlated with the estimates of h^2_{GCTA} with uncommon SNPs, having correlation coefficients (r) of 0.957. All estimates of h^2_{GCTA} with uncommon SNPs were within the confidence interval for the mean h^2 estimates and the slope of the regression equation did not differ from one (1.09 ± 0.09). The intercept of the equation did not differ from zero (0.030 ± 0.04), indicating no bias of h^2_{GCTA} with uncommon SNPs compared to

realized heritability. Estimates of h^2_{GCTA} from only common SNPs (11,941 SNPs with MAF $> 10\%$) were also close to averages of realized heritability estimates and highly correlated with h^2_{GCTA} with uncommon SNPs (r = 0.99, $P<0.001$) (Table 2). The estimates based on common SNPs alone were lower than those obtained from the uncommon data set (average reduction = 0.04 for the four traits).

Estimates of h^2_{GCTA} using 20,000 or 10,000 randomly sampled SNPs were very close to those calculated with uncommon SNPs (Figure 1). However, estimates of h^2_{GCTA} using only 2500 SNPs were lower for all traits. The range of estimates was from 0.058 to 0.12 lower for whole wet weight and shell height, respectively. In contrast, removing the 1000 SNPs that GWAS identified as mostly closely associated with phenotypic variance in the data had only minor effects on h^2_{GCTA} estimates (Table 2). This result reinforces that the GCTA method does not require causative SNPs to be genotyped for accurate estimates of heritability, as long as SNP density is high enough to accurately capture fine-scale relatedness.

Estimates of h^2_{GCTA} for each chromosome separately for the four traits were distributed across several chromosomes (Figure 2). When estimates of chromosome-wise heritability were regressed against the chromosome lengths, we noticed that significant linear trends (P=0.005245, P=0.03183, P=0.02966 and P=0.03941) for longer chromosomes to explain larger proportions of the variance for shell length, shell height, shell width and whole wet weight. Chromosome-wise heritability estimates in Figure 2 suggested that the genetic contribution to growth complex traits is scattered

across the genome and driven by many loci with small effect rather than a few causal loci with a large effect.

4. Discussion

There have been a number of studies which have predicted realized heritability of growth in bivalves. Wada (1988) reported response of the Japanese pearl oyster (*Pinctada fucata martensii*) to selection for several shell traits. After three generations of selection, realized heritability was estimated at 0.47 for shell width and 0.35 for shell convexity. However, realized heritabilities for shell width in the Catarina scallop (*Argopecten ventricosus*) was only about 0.2 (Ibarra et al., 1999). Realized heritabilities for shell height in the Bay scallop (*Argopecten irradians irradians*) and the Japanese scallop (*Patinopecten yessoensis*) were about 0.35 and 0.27, respectively (Zheng et al., 2006; Liang et al., 2010). The above heritability estimates using the traditional approaches were generally in the range of 0.2-0.5 and existed big differences even for the same traits, suggesting that heritability is environmentally dependent in bivalves. Using traditional approaches for estimating heritability is more challenging if organisms are grown or reared in natural settings, especially for bivalves. As one of alternative methods, the GCTA method can estimate the distant genetic relationship between unrelated individuals using SNP data and then using the estimated relationship to infer heritability from the proportion of phenotypic variance explained by genotyped SNPs (Yang et al., 2011). This study is, to our knowledge, the first to estimate realized

heritability for growth in scallop using the GCTA method with 2b-Rad SNPs in a selective breeding programe. The heritabilities of 0.42 (S.E. 0.09) for shell length, 0.47 (S.E. 0.07) for shell height, 0.54 (S.E. 0.11) for shell width and 0.28 (S.E. 0.03) for whole wet weight, which were estimated with uncommon SNPs using the GCTA method here, were strongly correlated (r = 0.957) with realized heritability estimated using the traditional approaches and in the moderate-to-high range, in line with values previously obtained for growth rates in bivalves (Van Vleck and Oltenacu, 2006). For shell width, our estimate of heritability was much bigger than that estimated by Ibarra et al. (1999) for Catarina scallop and similar to the heritability estimated by Wada (1988) for Japanese pearl oyster. What kind of environmental variable is affecting shell width is not known for certain. However, from triploid studies in scallops, it is known that sterility results in increased adductor muscle weight and increased shell width or convexity (Tabarini, 1984). Ibarra et al. (1999) reported that shell width in scallops is a trait associated with maternal quality: a large shell width in a non-sterile individual would indicate a greater ability for storing energy reserves for maturation and a better larval output (number and quality).

Our study demonstrated that GCTA-based estimates of heritability are relatively robust to SNP number (Figure 1). For the four traits, we examined h^2_{GCTA} estimates obtained with 10,000 SNPs or 20,000 SNPs were similar to h^2_{GCTA} estimates with 26,471 SNPs obtained by 2b-Rad sequencing, which is one of efficient reduced-representation approaches. Because it means that the number of SNPs needed to obtain

robust estimates of h^2 can be assayed using the reduced-representation approaches (such as GBS, Rad or 2b-Rad), which are both less expensive and faster than full-genome sequencing, especially for non-model species. Our study showed that causative SNPs or SNPs that are in linkage disequilibrium with causative SNPs had little effect on obtaining reliable estimates of h^2 (Table 1), suggesting that causal SNPs is not necessary for accurate estimates of h^2. Similarly, Meuwissen and Goddard (2010) showed that at higher marker density, only about an extra 3% increase in prediction accuracy by inclusion of the causative SNPs. While h^2_{GCTA} estimates appear robust to high SNP density and exclusion of causative SNPs, h^2_{GCTA} estimates are affected by assaying only common SNPs. Our study found that h^2_{GCTA} estimates using only common SNPs were lower than those conducted with uncommon SNP data set (Table 1). This results are consistent with the reported study that using 250 k common frequency SNPs, they explained 45% of the phenotypic variance of human height (Yang et al., 2010). Despite lower estimates, estimates of h^2_{GCTA} using common SNPs (MAF >10%) were tightly correlated with h^2_{GCTA} estimates conducted with uncommon SNPs (Table 1), indicating appropriate corrections could be used to estimate heritability using only common SNPs.

GCTA-based estimates of chromosome-wise heritability were used to provide information about the genetic architectures of scallop growth traits. Clearly, several variants across different chromosomes contribute to scallop growth trait variation. The finding that the contribution by an individual chromosome is significantly correlated to

its length suggests that on each chromosome might be many variants with a small to moderate effect size rather than a few variants with major effect. This hypothesis is also supported by results of the marker-associated selection for complex traits in domestic animals, which reported that variation in the traits does have a large genetic component, but so far most of the variants with small to moderate effects have not been identified in genetic association studies because of inadequate statistical power (Goddard and Hayes, 2009). GCTA, one of genomic selection methods, can be used to overcome the deficiencies of marker-associated selection, which is likely to lead to a low number of SNPs with validated associations that explain a small proportion of the genetic variance in the trait, for estimating heritability by using a genome-wide panel of dense markers so that all QTLs are in linkage disequilibrium with at least one marker.

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6. Author Contributions

Y.W., Z.B. and S.W. conceived and designed the study. H.G., Q.Z and Y.L. were involved in preparation of 2b-RAD libraries for sequencing. H.G. and P.L. conducted the major part of the bioinformatics analysis. Y.W., Z.C., P.L., S.W. and Z.B. drafted the manuscript. All authors read and approved the final manuscript.

7. Data accessibility

Phenotype and sequence data are available from :" http://mgb.ouc.edu.cn/cfbase/html/download.php ".

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Figure Legends

Figure 1. Box-and-whisker plots of h^2_{GCTA} estimates from 100 samples each made with the 538 scallops at 24 months in the selected groups and 2,500, 10,000, 20,000 or 26,471 SNPs for shell length, shell height, shell width, and whole wet weight.

Figure 2. Chromosome-wise heritability estimation for scallop growth traits. The numbers show the heritability point estimates of each chromosome. The solid lines plot the linear regression of chromosome-wise heritability against chromosome length; the dotted lines show 95% CI.

Figure 1

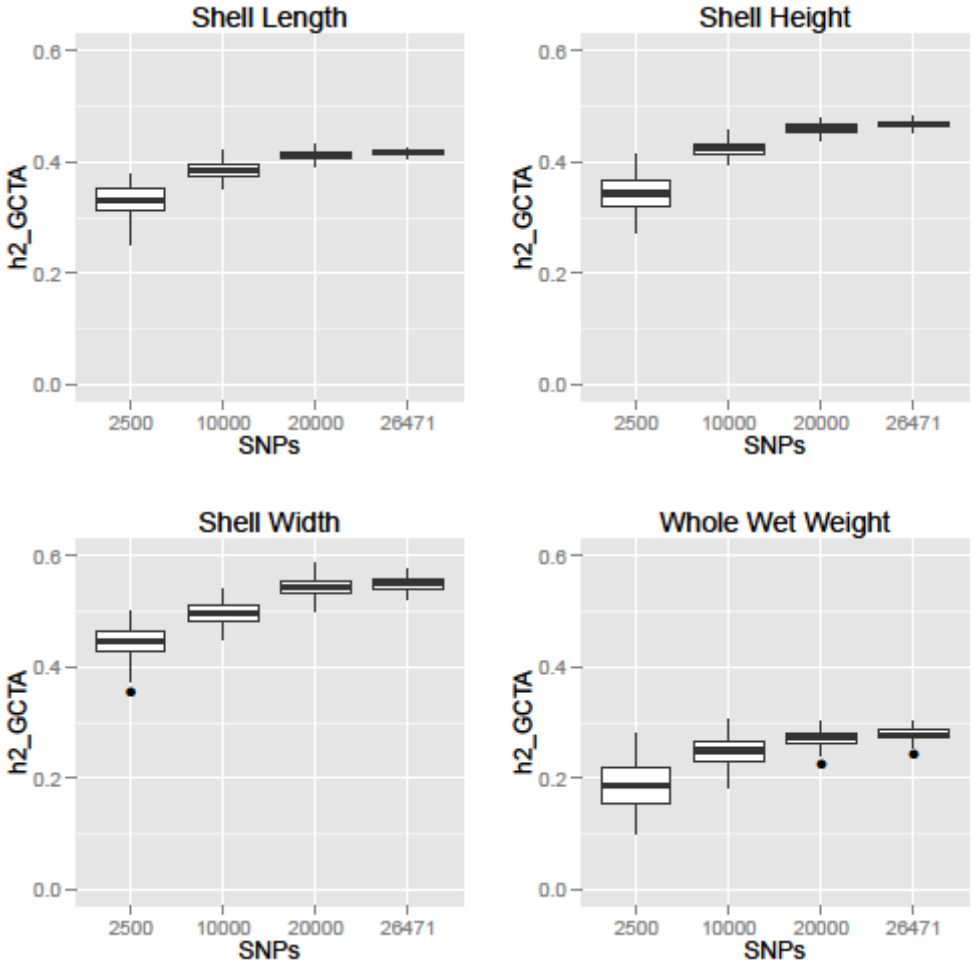

Figure 2

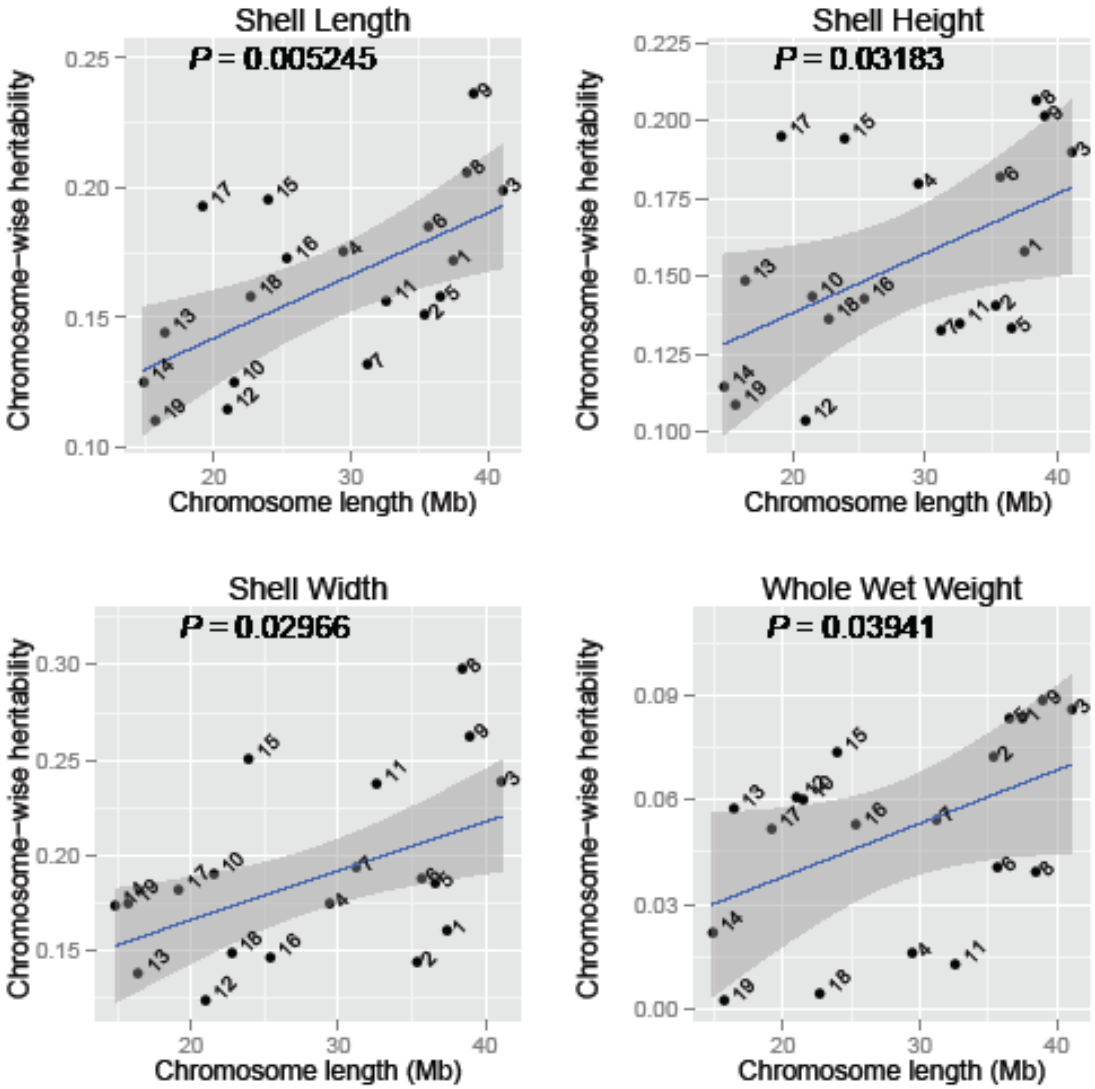

Table 1：Mean size (shell length, mm; shell height, mm; shell width, mm; whole wet weight, g), standard deviation (sd), results of t-tests, response to selection (R) and realized heritability (h^2) in the selected and control group at different ages (months) for four traits.

Trait	Age	Selected			Control			t_s	P	R	h^2
		N_s	$\bar{x}_s$	sd_s	N_c	$\bar{x}_c$	sd_s				
Shell Length	8	100	38.35	2.33	100	37.12	2.56	3.55	<0.001	1.23	0.29
	12	100	45.37	3.21	100	43.21	4.18	4.09	<0.001	2.16	0.31
	18	100	62.03	4.32	100	58.78	5.26	4.77	<0.001	3.25	0.37
	24	100	71.82	4.87	100	68.13	5.51	5.02	<0.001	3.69	0.41
Shell Height	8	100	33.63	1.19	100	32.75	1.62	4.38	<0.001	0.88	0.33
	12	100	40.59	2.94	100	38.85	2.78	4.30	<0.001	1.74	0.38
	18	100	58.92	3.61	100	55.34	4.59	6.15	<0.001	3.58	0.47
	24	100	68.13	4.69	100	63.32	6.51	5.99	<0.001	4.81	0.45
Shell Width	8	100	9.34	1.13	100	8.59	1.19	4.57	<0.001	0.75	0.36
	12	100	11.51	1.28	100	10.49	1.32	5.55	<0.001	1.02	0.45
	18	100	17.66	1.73	100	15.78	2.13	7.36	<0.001	1.88	0.51
	24	100	22.43	2.28	100	19.57	3.12	7.40	<0.001	2.86	0.54
WholeWet Weight	8	100	7.65	0.82	100	7.24	1.04	3.09	<0.001	0.41	0.25
	12	100	12.83	1.55	100	11.91	1.97	3.67	<0.001	0.92	0.29
	18 24	100	25.86 47.26.8	4.72	100 10	23.63	5.28	3.15 3.26	<0.001	2.23	0.26

		100	18	9	0	43.94	7.16		<0.001	3.24	0.28

Table 2： Mean realized heritability of different ages h^2 and Estimates of h^2_{GCTA} for four traits using uncommon SNPs with MAF ＞ 2%, common SNPs with MAF ＞ 10%, and with the top 1,000 major SNPs masked (MAF＞ 2%). Standard errors for h^2_{GCTA} estimates are in parentheses.

Trait	h^2	h^2_{GCTA} (S.E.) MAF > 2%	h^2_{GCTA} (S.E.) MAF > 10%	h^2_{GCTA} (S.E.) Major SNPs masked
Shell Length	0.35	0.42(0.09)	0.39(0.08)	0.42(0.08)
Shell Height	0.41	0.47(0.07)	0.43(0.09)	0.46(0.07)
Shell Width	0.54	0.54(0.11)	0.50(0.12)	0.53(0.11)
Whole WetWeight	0.27	0.28(0.03)	0.26(0.05)	0.28(0.03)

Highlights

- To compare the estimates of GCTA-based heritability with the estimates of realized heritability obtained from the empirical method in selective breeding.
- To evaluate how estimates of GCTA-based heritability are affected SNP density and minor allele frequency (MAF).
- To examine the effects excluding putatively causal SNPs has on GCTA-based heritability estimates.
- To assess whether the genetic contribution to growth is driven by a few SNPs with a large effect or many SNPs with small effect.